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MEETING REPORT

Animal Models for Radiation Injury, Protection and Therapy

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Current events throughout the world underscore the growing threat of different forms of terrorism, including radiological or nuclear attack. Pharmaceutical products and other approaches are needed to protect the civilian population from radiation and to treat those with radiation-induced injuries. In the event of an attack, radiation exposures will be heterogeneous in terms of both dose and quality, depending on the type of device used and each victim's location relative to the radiation source. Therefore, methods are needed to protect against and treat a wide range of early and slowly developing radiation-induced injuries. Equally important is the development of rapid and accurate biodosimetry methods for estimating radiation doses to individuals and guiding clinical treatment decisions. Acute effects of high-dose radiation include hematopoietic cell loss, immune suppression, mucosal damage (gastrointestinal and oral), and potential injury to other sites such as the lung, kidney and central nervous system (CNS). Long-term effects, as a result of both high- and low-dose radiation, include dysfunction or fibrosis in a wide range of organs and tissues and cancer. The availability of appropriate types of animal models, as well as adequate numbers of animals, is likely to be a major bottleneck in the development of new or improved radioprotectors, mitigators and therapeutic agents to prevent or treat radiation injuries and of biodosimetry methods to measure radiation doses to individuals.

INTRODUCTION

On May 25–26, 2004, the Division of Allergy, Immunology and Transplantation (DAIT), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) sponsored a workshop on Animal Models for Radiation Injury, Protection and Therapy. The main goals of this workshop were to identify the most appropriate animal models to evaluate radioprotectors and therapeutic agents (including both mitigators and treatments), to develop accurate and user-friendly biodosimetry methods, and to identify gaps in the infrastructure needed to advance mechanistic studies and product development for protection against and mitigation and treatment of radiation injury. The workshop was divided into three sessions that addressed current and future radioprotectors and therapeutic agents, characterization of radiation injury to organ systems, including biodosimetry methods, and application of specialized animal models for assessment of radiation injury, protection and therapy. The presentations were followed by breakout sessions that mirrored the primary sessions in subject matter. The main goal of each breakout session was to discuss available resources, focusing on the most appropriate animal models, and to identify gaps and opportunities to advance discovery and development of radioprotectors and therapeutic agents. This meeting report provides an overview of the topics discussed and the recommendations put forth to improve protection against and mitigation and treatment of radiation injuries resulting from the deliberate or accidental release of radioactive materials.

The current lack of radioprotectors and therapeutic agents that are safe, effective and approved for use in victims of an accidental or deliberate radiation exposure is a major problem in preparing for such events. Clinical differences exist between the carefully planned and monitored exposures to radiation during clinical therapy and exposures due to radiation accidents or attacks, in which the doses are uncontrolled and will likely range from low-level radiation to acute, lethal doses. New agents, approaches and regulatory processes are needed to provide products for the protection and treatment of large numbers of casualties in a reasonable time. Most of the United States Food and Drug Administration (FDA)-approved therapeutics can be admin-

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istered only for limited indications. For example, the immune-stimulating cytokine Neupogen[R] [Amgen Inc. trademark for Filgrastim; granulocyte colony-stimulating factor (G-CSF)] is approved for use in immune-suppressed cancer patients to decrease the incidence of infection, but currently it can only be used off-label in victims of radiation accidents or attacks. The ideal radioprotector or therapeutic agent must be safe for all populations at risk of radiation exposure, even with repeated doses (as needed), easily administered, rapidly effective, and chemically stable. It also needs to be simple and inexpensive to manufacture. Recently, the FDA developed new guidelines for efficacy testing of new therapeutic agents and protectors against radiation/nuclear, biological or chemical threats, which, for ethical reasons, cannot be tested in challenge studies in humans (67 FR 37988, amended parts 314 and 601, http://www.fda.gov/cber/rules/humeffic.pdf). These guidelines require that efficacy studies be conducted in two relevant animal species and that safety and pharmacokinetic studies be conducted in humans prior to FDA approval. The FDA accepts animal efficacy data as evidence of efficacy only in those cases where:

1. there is a reasonably well-understood pathophysiological mechanism of toxicity for both the harmful agent and the prevention of this toxicity by the product;
2. efficacy has been substantiated in more than one species;
3. the animal studies have used end points that clearly relate to the desired benefit in humans; and
4. the animal studies have shown responses that are predictive of human responses in regard to pharmacokinetics, safety and efficacy.

These FDA guidelines will permit identification and testing of radioprotectors and therapeutic agents for use against radiation exposure due to an accident or attack. It is important for investigators to begin discussions with their institutional animal welfare committees and the FDA early in the research process to ensure that the correct animal models are being used and that the questions being addressed will provide the appropriate data for FDA approval.

HEMATOLOGICAL EFFECTS

The hematopoietic system is highly sensitive to ionizing radiation. Doses of 2 Gy and above cause decreased lymphocyte counts and immune suppression, making victims susceptible to secondary infections. Therefore, protection or reconstitution of the hematopoietic and immune systems is a major concern in the development of radioprotectors and therapeutic agents.

Radioprotectors can be divided into six categories: pharmacological agents, nutraceuticals, growth factors and cytokines, immune modulators, gene or cell therapy, and physical devices. Amifostine, a pharmacological agent (*1*), is approved by the FDA for use in cancer patients for prevention of specific side effects of radiation. It is an effective radioprotector in animal models; however, at the high doses required to provide radioprotection, amifostine induces significant side effects that decrease physical and mental performance and make it unsuitable for first responders, medical staff, military personnel, and others who must be capable of rapid and effective action in an emergency. Efforts are under way, using mouse and non-human primate animal models, to reformulate amifostine to decrease its toxic side effects and improve delivery methods while maintaining its radioprotective capability. Alpha-tocopherol succinate (a vitamin E derivative, nutraceutical) exhibits marginal but significant radioprotective properties in small animal models (*2–4*). Additional studies in large animals and with different formulations are needed to fully evaluate its potential radioprotective capabilities. 5-Androstenediol (5-AED) is being developed as a radioprotector and mitigator through a collaboration of AFRRI (Dr. Mark Whitnall) and Hollis-Eden Pharmaceuticals. 5-AED is a natural steroid that displays extremely low toxicity and androgenicity. In γ-irradiated mice, subcutaneous injection of 5-AED enhances survival and stimulates hematopoiesis, elevating numbers of circulating neutrophils, monocytes, natural killer cells, erythrocytes and platelets (*5*). The compound has also been shown to mitigate radiation-induced neutropenia in dogs and non-human primates.

As with radioprotectors, therapeutic agents (mitigators and treatments) can be divided into four different groups: cytokines and growth factors, cell replacement, clinical support, and antibiotics. There is a need for the parallel development of radioprotectors and therapeutics from the different classes of potentially useful compounds, as noted by Dr. Thomas Seed.

In terms of the development of drugs for radiation injury, Dr. Thomas MacVittie noted that the definition of an effective treatment strategy for enhanced survival from the hematopoietic syndrome will depend upon available treatment protocols, drugs/growth factors, and the prevailing characteristics of the radiation accident. Namely, the radiation environment is likely to be ill-defined and uncontrolled, and the exposure may be nonuniform, partial-body, and of variable dose rate and exposure duration. Furthermore, the time between exposure and treatment is usually not optimal, and it may be difficult to establish an accurate absorbed dose. He proposed that there is only one treatment strategy available now for severely irradiated individuals that is directed at the critical outcome of radiation-induced myelosuppression, namely, neutropenia and prevention of infection. The strategy's two components are aggressive supportive care and administration of recombinant cytokines or myeloid growth factors, such as granulocyte colony-stimulating factor (G-CSF), granulocyte/monocyte colony-stimulating factor (GM-CSF) or pegylated G-CSF, as soon as possible after irradiation. This proposed treatment scheme is supported by a consistent and substantial body of evidence from experiments on severe radiation-induced myelosuppression and lethality in dogs and rhesus mon-

keys. Each of these cytokines has completed phase I and subsequent clinical trials and is FDA-approved for treatment of chemotherapy-induced neutropenia. In addition, G-CSF (Neupogen, Amgen) is in the National Stockpile and, if needed, this drug can be used under Investigational New Drug (IND) status to treat victims of radiation exposure. Further studies are required to gain FDA approval for use of these cytokines as conventional treatments for acute radiation syndrome and hematological effects.

Results from Dr. Seed's laboratory show that G-CSF plus interleukin 11 (IL11) provides broad-spectrum protection after irradiation by stimulating the proliferation of hematopoietic progenitor cells and the reconstitution of neutrophil, monocyte, erythrocyte and platelet populations. Hematopoietic cell transplantation also holds promise as a potential treatment for victims of radiation exposure. Dr. John Chute's laboratory has advanced the concept of reconstituting human hematopoietic cells with autologous radioresistant stem cells (*6*). In their model system, radioresistant hematopoietic stem cells are recovered from the bone marrow of lethally irradiated mice and expanded *ex vivo* for 10 days with endothelial feeder cells or cytokines. Stem cell recovery is enhanced by co-culture with endothelial cells, compared to culture with cytokines alone. The progeny of these *ex vivo* cultures provide multi-lineage hematopoietic cell reconstitution in transplanted recipient mice. These studies suggest that autotransplantation of bone marrow stem cells expanded *ex vivo* may be a potential therapy for victims of myeloablative injury caused by high doses of ionizing radiation.

Recent advances in genomics and genetic engineering provide an opportunity to create novel animal models that more closely mimic human responses. Dr. Leonard Schultz's group (unpublished results) is one of several teams around the world (*7*) that are developing novel mouse models to study human hematopoietic stem cell function *in vivo*. The new xenograft models are an improvement over existing mouse xenograft models, which sustain only limited development and maintenance of human lymphoid cells and rarely produce immune responses. These new models support the development of functional human T cells, B cells, dendritic cells, and monocytes, providing valuable models to study human hematopoietic stem cell differentiation and immune reconstitution, as well as to test methods to enhance immune recovery after radiation exposure.

GASTROINTESTINAL EFFECTS

With intensive supportive therapies, currently available protectors and therapeutic agents that decrease radiation-induced hematological injury will allow many patients to survive the hematological crisis, which was once inevitably lethal to victims of radiation accidents and incidents receiving moderate-dose radiation exposure. For these people, the degree of damage to the gastrointestinal (GI) system becomes the main determinant for survival. Gastrointestinal responses to radiation have been studied in a variety of animal models. As noted by Dr. Martin Hauer-Jensen, mice and rats are the preferred species for ethical, biological, logistic and economic reasons. In terms of effects on GI function, responses of the rat intestine to radiation exposure more closely resemble those of humans, compared to mice. Although mice tend to respond differently, genetically modified mouse models may provide greater opportunity for mechanistic studies of radiation-induced intestinal damage, due to the ease of development of mouse models with specific genotypes. Choice of the most appropriate animal model will depend on the desired end points. Small animals are most appropriate for mechanistic studies of radiation-induced injury, whereas other animals (such as the ferret, pig or dog) may be more appropriate for analysis of effects such as emesis and for the evaluation of novel protectors and therapeutic agents.

Intestinal radiation injury occurs as a consequence of many concurrent and sequential pathophysiological events, including the induction of injury by reactive oxygen species (ROS), enterocyte depletion, mucosal barrier breakdown, mucositis with secretory diarrhea, bacterial translocation across the wall of the gut, and adverse tissue remodeling. Many interventional strategies, such as ROS scavengers, antioxidants, epithelial growth factors, cytokines and immune modulators, show efficacy in preclinical studies, but few can be recommended due to safety issues or treatment requirements, and none are in routine clinical use. A current focus of Dr. Hauer-Jensen's laboratory is developing interventions that preserve or restore endothelial function and minimize intestinal radiation toxicity. For example, statins, which are currently used to treat high cholesterol, exhibit vasculoprotective effects that are unrelated to lipid lowering and suggest a role in the regulation of thrombomodulin. Intestinal radiation toxicity involves dysregulation of the thrombomodulin-protein C pathway, which results in increased thrombin production, platelet aggregation, and increased transforming growth factor beta (TGFB) expression as well as inflammation and increased tumor necrosis factor alpha (TNFA) production. Data from Dr. Hauer-Jensen's laboratory show that statins increase endothelial thrombomodulin activity and ameliorate intestinal radiation toxicity in a rodent model. Statins may also have a role in protection against infections that may accompany radiation-induced GI damage. Preliminary clinical and animal studies suggest that statins may reduce susceptibility to and decrease mortality from sepsis[2] (*8*).

SECONDARY INFECTION

Infection is the primary cause of death from doses of ionizing radiation that induce hematopoietic and GI syndromes. The use of broad-spectrum antibiotics combined

[2] VA population VISN16 Data Warehouse.

with clinical support increases survival of acutely irradiated, severely neutropenic dogs by 50% during the first 60 days after irradiation (*9, 10*). Dr. Thomas Elliott uses mouse models to study susceptibility to and treatment of radiation-induced infection. High-dose radiation with accompanying GI damage results in bacterial translocation from the intestines to other sites in the body and increases mortality. Currently, antibiotics are prescribed for radiation victims as symptoms develop. An FDA-approved antibiotic regimen for optimal prevention or treatment of infection after radiation injury is needed. Antimicrobial therapy in such patients should be directed against a range of microorganisms that cause polymicrobial sepsis including both gram-negative and gram-positive bacteria; concomitant antiviral treatment may also be required. The antimicrobial therapy needs to be chosen carefully because an inappropriate antimicrobial agent can actually decrease survival. Antimicrobials such as quinolones that do not target beneficial intestinal anaerobic bacteria are preferable because they limit translocation of pathogenic bacteria. Dr. Elliott's studies indicate that innate immune boosters such as β-1-3-glucan significantly enhance survival of mice when given in combination with antimicrobial therapy. Dr. Elliott's studies also suggest that antimicrobial research should be directed at broad-spectrum reagents that retain therapeutic stability during prolonged storage and should include agents that boost both innate and adaptive immunity.

RENAL EFFECTS

Radiation-induced renal damage can manifest 8 months to 20 years after exposure. Experimental and clinical data suggest that chronic renal failure is observed if a bilateral dose exceeds 4–5 Gy when hematological lethality is avoided by partial shielding or medical intervention (*11–13*). The mouse is relatively resistant to radiation-induced renal damage and transgenic mouse models may not be appropriate, since the outcomes seen in these models do not correlate well with the clinical outcomes seen in humans. The rat, dog, pig and non-human primate exhibit physiological and histopathological changes similar to those seen in people with radiation-induced renal injury, although the time between irradiation and the development of renal disease varies. Rats, dogs and pigs exhibit disease much sooner than humans but still require months of follow-up to determine the long-term efficacy of an intervention. Non-human primates are not practical models because the time course for the development of disease is similar to that for humans; approximately 6 years of follow-up are needed to evaluate an intervention.

Dr. John Moulder noted that the classic dogma in this field, which states that radiation nephropathy is untreatable and is due only to a reduction in clonogenic cell survival of proximal tubule epithelial or endothelial cells, does not correspond with recent clinical and experimental data. For example, studies in rats and clinical trials in human patients show that antagonism of the renin-angiotensin system with angiotensin-converting enzyme (ACE) inhibitors or angiotensin II (AII) receptor blockers can stabilize renal function (*11, 14, 15*) when used as therapeutics. ACE inhibitors appear to function by stopping injury progression; they do not seem to repair existing damage. AII inhibitors may work a bit better than ACE inhibitors to prevent disease progression, though more data are needed. The ACE inhibitor Captopril is currently in NCI-supported clinical trials at the Medical College of Wisconsin to test its ability to prevent renal damage in bone marrow transplant recipients. Major bottlenecks in the development of improved therapeutic agents for radiation-induced renal injury include a lack of detailed understanding of the pathophysiology of radiation-induced renal damage or of the underlying mechanisms of efficacy of proven interventions such as amifostine, ACE inhibitors, or AII blockers (*16, 17*). Although these issues were not discussed at the workshop, recent studies point to changes in gene expression (*18*), glomerular permeability (*19*), and markers of DNA oxidation (*20*) as possible early markers for late renal injury; these results need to be validated. This knowledge would aid identification of early surrogate markers that accurately predict the development of late disease, thereby accelerating the screening of novel or improved treatments in current animal models by decreasing the follow-up time required.

CENTRAL NERVOUS SYSTEM EFFECTS

In mammals, new neurons are produced within the hippocampal dentate gyrus of the brain throughout life. Ionizing radiation leads to an acute and dose-related loss of neural precursor cells, persistent changes in neurogenesis, and hippocampal cognitive impairment (*21–23*). Although the complete mechanism of action of radiation-induced central nervous system (CNS) damage has not been defined, *in vitro* and *in vivo* studies suggest that local inflammation and oxidative stress cascades (including production of ROS) are involved. Dr. John Fike's group, using rat and mouse model systems, has identified a radiosensitive neuron precursor population in the brain; reductions in this population may contribute to the observed neurocognitive defects. While the animal studies are likely to be valuable for determining the mechanisms of radiation-induced CNS damage, it should be noted that differences between animals and humans do exist and must be considered in designing and interpreting experiments. For example, rat brains are much more radioresistant than human brains (*24*). Dr. Fike's group overcomes this difference by irradiating the rats with doses that are higher than the clinically relevant human dose but that produce physiological effects similar to those observed in human patients. Radiation induces dose-dependent increases in ROS production, neuronal apoptosis, and levels of inflammatory cells in the brain (*25*). Administration of lipoic acid to the mice for 1 week reduces ROS-induced apoptosis and increases the

number of neural precursors in the hippocampus. Adoptive transfer of normal neural precursors into irradiated mice does not limit the radiation effects; one explanation is that radiation alters the entire neuronal environment, thereby affecting neurogenesis. An increase in microglial cells observed postirradiation suggests the involvement of inflammatory processes. Behavioral training and testing enhance neurogenesis in irradiated mice compared to untested irradiated animals. These studies suggest that pharmacological agents, such as lipoic acid or anti-inflammatory compounds, and mental exercise may lessen the consequences of radiation-induced CNS damage. However, a clearer understanding of the pathogenesis of radiation-induced CNS damage is needed to foster development of successful protective and therapeutic strategies.

ORAL EFFECTS

Oral mucositis is a severe consequence of exposure to ionizing radiation that can occur days to weeks after exposure. The mechanism of action appears to be through induction of a reactive oxygen species-mediated hypoxia and of a cytokine cascade that enhances clonogenic death of cells in the mucosal basal epithelium. A promising radioprotector against oral mucositis is recombinant human keratinocyte growth factor (KGF, commercial name Palifermin). Dr. Wolfgang Dörr's studies using a mouse model demonstrate that administration of KGF from day -4 to $+5$, in a single radiation exposure protocol, results in significant reduction of oral mucositis (*26*). KGF appears to prevent the development of oral lesions if administered before or soon after radiation exposure. Thus it can act as a radioprotector or mitigator. However, administration of KGF after the onset of oral lesions (approximately day 10 postirradiation) does not prevent further damage or promote healing of existing oral lesions and therefore is not a therapeutic agent. KGF induces epithelial cell proliferation and differentiation in mucosa, type II pneumocytes, and salivary glands. The protective effects of KGF are seen within a large dose range in animal studies, suggesting that a standard dose per person may be defined through additional studies. Palifermin has been used in phase III clinical trials to prevent oral mucositis in non-Hodgkin's lymphoma patients undergoing radiation therapy (*27*) with promising results. Grade 3 and 4 oral mucositis was reduced significantly, resulting in an increased prevalence of Grade 1 and 2 oral mucositis. While these studies are promising, more research is needed to establish whether the protective and mitigating effects of KGF can be used for victims of radiological accidents or deliberate attacks.

Dr. James Mitchell presented data on a class of radioprotectors developed in his laboratory. Nitroxides are stable free radicals that protect against toxicity induced by superoxide, hydrogen peroxide, organic hydroxides, ionizing radiation, or DNA-damaging anticancer agents. When administered at non-toxic concentrations, nitroxides exhibit effective antioxidant properties, both *in vitro* and *in vivo* (*28, 29*). Administration of Tempol (4-hydroxy-2,2,6,6-tetramethylpiperridine-*N*-oxyl) 10 min before radiation exposure protects against radiation-induced lethality in mice (*30*), alopecia in rodents and humans (*31*), and salivary gland dysfunction in mice (*32*). In contrast, Tempol does not protect tumors against radiation-induced regrowth delay after either single or fractionated radiation treatment. Dr. Mitchell attributes the radiation protection by nitroxides to intracellular scavenging of radiolytically generated free radicals, particularly carbon-centered radicals. Interestingly, only the oxidized form of the nitroxide protects against radiation damage; the reduced form (hydroxylamine) does not. Novel electron paramagnetic resonance redox imaging studies have shown that nitroxides are rapidly reduced to hydroxylamines in mice and that reduction is faster in tumors compared to normal tissues. By employing functional imaging, the rates of nitroxide reduction in tumor compared to normal tissue can be obtained. Such knowledge may offer the possibility of administering nitroxides prior to radiation exposure to selectively ameliorate radiation-induced damage to normal tissue.

PULMONARY EFFECTS

Radiation damage to the lung results in acute and chronic inflammation that can lead to fatal lung fibrosis. Dr. David Brizel and colleagues have developed a rat model using single-fraction and multiple-fraction ipsilateral whole-lung irradiation to evaluate the clinical regimen used for the treatment of lung cancer. Data using this model show that KGF (*33*), superoxide dismutase (SOD) mimetic agents, and a TGFB receptor antagonist improve breathing rates and reduce the severity of lung fibrosis when administered soon after irradiation. These compounds target different steps in the development of lung fibrosis. KGF appears to decrease integrin expression on epithelial cells, resulting in a diminution of the TGFB cytokine cascade and the number of activated alveolar macrophages thought to contribute to lung fibrosis. SOD mimetics decrease expression of hypoxia-inducible factor 1 (HIF1), which is up-regulated in hypoxic tissue after lung irradiation and regulates the transcription of hypoxia-responsive genes, and also decrease plasma levels of TGFB.

Dr. Joel Greenberger used manganese superoxide dismutase (MnSOD) plasmid liposomes to protect against acute radiation mucositis in the oral cavity and oropharynx and against lung fibrosis in C3H/HeJ and C57BL/6J mouse models, respectively (*34–36*). In the C57BL/6J mouse lung irradiation model, lung fibrosis develops at 120–140 days postirradiation. Dr. Greenberger demonstrated that fibrosis is induced in part by migration of bone marrow-derived macrophages and fibroblasts into the lung. MnSOD plasmid liposomes, given prior to irradiation, block free radical production in the lung and decrease the onset of late fibrosis.

Recent studies by Dr. Richard Phipps and colleagues

point to another possible target for the prevention of lung fibrosis: peroxisome proliferators-activated receptor gamma (PPARγ) (unpublished results). This transcription factor is found within platelets and human fibroblasts and is a key regulator of adipocyte differentiation and inflammation. Small molecule inhibitors of PPARγ have been used to treat diabetes (Rosiglitazone) and to reduce inflammation by decreasing platelet activation and aggregation and release of CD40 ligand (CD40L) and other bioactive mediators of inflammation. Data from Dr. Phipps's laboratory show that, in a mouse model system, PPARγ agonists also prevent TGFB from driving fibroblasts to differentiate into myofibroblasts, the hallmark of fibrosis, and reduce lung inflammation. Human fibroblasts express CD40, a receptor found on most innate and adaptive immune cells that activates these cells upon binding to CD40L expressed on T cells and platelets (*37*). Fibroblasts exposed to CD40L produce a variety of pro-inflammatory mediators, including cytokines, adhesion molecules, chemokines and extracellular matrix (*38*). Humans with radiation-induced lung fibrosis have high levels of CD40L in plasma and lung lavage samples. Using a mouse model of radiation-induced lung injury and fibrosis, Dr. Phipps showed that administration of monoclonal anti-CD40L antibody dramatically reduces lung inflammation and fibrosis in mice. However, this method cannot be used in humans because human platelets also express CD40L and the monoclonal antibody induces platelet aggregation and clot formation. As an alternative approach, Dr. Phipps is testing small molecule agonists of PPARγ that might prevent the development of lung fibrosis in humans.

INTERNALLY DEPOSITED RADIONUCLIDES

Internally deposited radionuclides also pose a serious threat in certain radiological or nuclear attacks. Dr. Bruce Boecker discussed the use of animal models to study dosimetry, biological effects, and therapy for insoluble inhaled radioactive materials, using ^{144}C in fused aluminum particle (FAPs) to compare the model systems. Lung retention of FAPs is similar in humans and dogs but is different in mice and rats, which clear the compounds more quickly than the larger species (*39, 40*). Therefore, dogs are valuable models for analysis of early-phase biological effects and for studying treatment strategies that might reduce the chronic radiation dose to the lung by improved radionuclide removal in the early postexposure period. A series of 10 bronchopulmonary lavage treatments, begun within 2 days after the inhalation exposure, effectively removes approximately 50% of the initial lung burden of an insoluble α-particle-emitting radionuclide such as $^{239}PuO_2$ or a β-particle-emitting radionuclide such as ^{144}CeFAP. One of the important current research challenges is to devise methods for increasing the rate of removal of insoluble radionuclides from the lung, thereby reducing the total dose and the associated insoluble radionuclide forms from the lung. Chelating agents can be a useful supplement to bronchopulmonary lavage for radionuclides inhaled in more soluble forms that are absorbed from the lung into the bloodstream.

Dr. Patricia Durbin-Heavey and colleagues are developing new actinide chelating agents composed of hydroxypyridonone metal binding units, most of which chelate several of the actinides (*41,* unpublished results). These new compounds are effective at low dose and can be given orally, unlike the $CaNa_3$-DTPA used currently. The efficacy of these new compounds has been tested in mice, and some have been tested in rats. The efficacy and the low toxicity for acute and chronic therapy observed in mice need to be verified in larger animals for further analysis of their therapeutic potential.

GENETIC FACTORS, CANCER DEVELOPMENT, AND NOVEL ANIMAL MODELS

The lifetime risk of developing a radiation-induced cancer is dependent on many factors including age at time of exposure, magnitude and type of exposure, and genetic variations in radiation sensitivity. The risk to children is about twice that for adults (*42*). In addition, there are populations with genotypes that induce radiosensitivity who may be at greater risk of developing cancer from low-dose radiation exposure than the general public. Evidence for radiosensitive populations comes from both human and small animal studies; current data suggest that 2–4% of the human population is unusually radiosensitive.

Dr. David Brenner and the group at Columbia University use ataxia telangiectasia mutated (Atm) heterozygous mice and the double heterozygote Atm/Brca1 (breast cancer 1) mice to study radiosensitive populations (*43,* unpublished results). Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by cerebellar ataxia, telangiectases, immunodeficiency, radiosensitivity and a predisposition to malignancies, including leukemia. There are a number of subtypes of AT; several of them are associated with mutations in the *ATM* gene. The ATM protein resides predominantly in the nucleus of a cell and normally functions to control cell growth rates. In addition, ATM is a key sensor of DNA damage and is involved in DNA repair; mutations in the gene appear to alter cell division and DNA repair mechanisms. Approximately 1–3% of the U.S. population is heterozygous for AT. Dr. Brenner's studies show that genetically based variations in low-dose radiosensitivity are real, but assessing their significance for the outcome of casualties in a radiation incident using epidemiological studies is difficult. Small animal models, such as the mouse, are valuable tools for the development of methods to monitor radiosensitive populations in the event of a deliberate or accidental radiation release.

Dr. Robert Ullrich's research focuses on identification of high-frequency/low-penetrance susceptibility genes and modifier genes that influence cancer risks after irradiation. A key step in studying heritable risks for cancer develop-

ment is determining which models are most appropriate. Data on the atomic bomb survivors provide a rich source for the identification of genetic factors that contribute to the development of leukemia and solid tumors after exposure to ionizing radiation. Genetically modified mice (e.g., transgenics, knock-ins, knockouts, inbred strains) are useful tools for determining the cellular and molecular mechanisms involved in cancer initiation and progression as well as other late effects of radiation damage. For example, Dr. Ullrich's group recently identified two BALB/c mouse strain-specific single nucleotide polymorphisms in the *Prkdc* gene, which encodes the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), an important component in the repair of DNA double-strand breaks by nonhomologous end joining (*44*, *45*). Genetic analyses of the altered Prkdc genotype suggest that the variant form of the gene product plays a role in initiating events, but not the progression, of radiation-induced mammary tumors. While *PRKDC* and *ATM* are two examples of genes that may alter radiosensitivity and cancer risk, many other genes are likely to play a role in an individual's risk of cancer after radiation exposure (as discussed in ref. *46*). Mouse models are useful for dissecting genetic factors that contribute to the initiation, clonal expansion, and progression of radiation-induced cancers.

BIODOSIMETRY

One of the major tasks of first responders and medical personnel is to determine the internal and external radiation doses received by victims. This critical information provides diagnostic information to the treating physicians and provides exposure assessments for individuals at the site of the incident, first responders, and medical staff. The current methods used for estimating the radiation dose include time to emesis, lymphocyte depletion kinetics, and cytogenetic changes in host cells. Dr. William Blakely and colleagues have developed the Biological Assessment Tool (BAT), a radiation casualty management software application (available at www.afrri.usuhs.mil), to facilitate medical recording and dose predictions based on clinical symptoms and available dosimetry. Dr. Blakely and colleagues also are developing novel cytological assays to quantify cells with chromosomal aberrations and are validating and optimizing other radiation-responsive nucleic acid and protein biomarkers to be used for rapid assessment of the radiation dose received by individual victims.

Similarly, Dr. Andrew Wyrobek is applying genome-scale surveys, including gene transcript microarrays and proteomics approaches, to identify biosignatures of radiation exposure and cell fate in mouse models and human cells (*47–49*). These studies are based on the knowledge that exposure to ionizing radiation induces complex changes in the expression patterns of gene transcripts and proteins and also produces modifications in proteins. His results demonstrate that specific genes are expressed in a dose-dependent, time-dependent and tissue-specific fashion in animal models and in human cells. Gene transcripts can also be assigned to specific biochemical pathways associated with cell fate after radiation exposure. Dr. Wyrobek is continuing the evaluation and validation of candidate molecular biomarkers of radiation exposure in human and animal models.

In addition to biological studies, computational models are also used during dosimetry estimates and risk assessment from radiation exposures. Dr. Keith Eckerman discussed the need for further development of computational models for radiation intake (lung, GI tract), absorption, excretion and dosimetry (*50*). Current models have been useful but may not accurately predict either the early dose rates or the non-linear effects seen at high intake doses. Biokinetic modeling is beginning to focus on physiology-based models that include human and animal data and consider variations in age and gender to better assess risks for individuals. Biological data are needed to improve the development of the models and the interpretation of their predictions. New models that incorporate parameters such as dose–response relationships for early effects, improved representation of early kinetics, and influence of therapy on biokinetics are needed to improve predictive capabilities and to allow assessment of individual risks and guide treatments. In addition to identifying novel biomarkers and developing improved modeling tools, there is a need to develop enhanced devices that measure biosignatures and that will allow for the rapid and accurate assessment of radiation exposure in the clinic and in the field. These issues were not discussed in detail at the workshop.

DISCUSSION

After the formal presentations, the participants broke into small groups to discuss three broad areas in relation to the presented research: available resources, such as assays, technologies, products, radiation sources, and animal colonies for collaborative use; the most appropriate animal models to address research or product development needs; and current and future opportunities to advance the areas of research or product development presented in each session, including required resources, data and infrastructure. Session chairs presented the discussion summaries and recommendations to the entire group. These recommendations are outlined below.

Resource Needs

1. Irradiation facilities, especially for large animals and for inhalation exposures.
2. Containment facilities for housing animals after exposure to inhaled, ingested or injected radionuclides (including long-lived radionuclides).
3. Human and animal tissue banks to facilitate broader access to valuable samples by the research community.

4. Centralized/core GLP and GMP facilities.
5. Access to specialized animal models such as transgenic and humanized rodents as well as large animals such as dogs, pigs, and non-human primates.
6. Core facilities for studying large animals.
7. Bioinformatics infrastructure, including a database of archived literature from radiation journals, AFRRI reports, National Laboratories (Lawrence Livermore, Oak Ridge, Lawrence Berkeley, etc.), and national/international meeting reports to provide access to existing animal and human data.
8. Improved screening capabilities: develop new or improved assays for identification and testing of new therapeutic targets, biodosimetry methods, and mechanistic studies; and create non-invasive assays for use in biodosimetry and safety/efficacy testing of radioprotectors, mitigators, and therapeutics in animal models and humans.

Animal Models

1. Most of the research to date on radiation effects, as well as the evaluation of radioprotectors, has been conducted using high-dose radiation or models of cancer radiotherapy. Additional studies of low-dose radiation exposure need to be conducted that more accurately mimic radiation exposures due to accidental or intentional release of radioactive materials.
2. Animal models are applicable to two stages of radiation research: mechanisms/discovery and validation. Each stage requires different animal models. Rodents are useful for studying mechanisms, while primate, dog, pig and possibly radiation therapy patients are useful for validation of the efficacy of protectors, mitigators and therapeutic agents.
3. Discussions with the FDA are needed to identify the appropriate, validated animal models and study end points that will be required to provide the appropriate data for FDA approval of new protectors, mitigators and therapeutics.
4. Multiple models are necessary for full analysis of any agent. Whole animals are necessary for some studies, but *ex vivo* or cultured cells will be appropriate and useful in others.
5. Develop animal models for assessment of radiation damage and treatments in special populations: These must consider the effects of age, gender, immune suppression, co-morbidity from infection or other underlying disease, and combined injuries.
6. New models are needed to provide a better understanding of the basic mechanisms of radiation damage; this will allow the rational design of protectors, mitigators and treatments. Existing specialized mouse models (e.g. cancer-prone strains, radiosensitive strains, genetically engineered strains) may also may be useful in such studies.
7. Primates provide an avenue for long-term studies of multiple syndromes or late/delayed effects, possibly being the most relevant to human exposure response. However, there are a limited number of laboratories with the expertise and capacity to use these models.
8. Availability of certain non-human primates may be limited, particularly specific-pathogen-free monkey species. Furthermore, they are very expensive and have a long time course for the development of pathology. Therefore, there is a need for novel biomarkers that allow early assessment of late effects in primates and a need for access to other large animal models (pig, dog) that model human responses with a shortened time course.
9. Archived and current human data from radiation accidents, occupational exposure, radiotherapy patients (early and late effects), and Hiroshima, Nagasaki and Chernobyl survivors are valuable; collection of data from these populations should continue, and the data should be made available to the broader research community for analysis.
10. Research gaps in the evaluation and development of improved protectors, mitigators and treatments include hematological data for irradiations other than high-dose-rate, low-LET radiation, identification and validation of new biomarkers for lethality and late effects (e.g. fibrosis, cancer), studies with partial-body exposures, co-morbidities, etc., and deployable technologies for triage and treatment decisions.

The National Institute of Allergy and Infectious Diseases, National Institutes of Health has been assigned the task of coordinating the NIH response to the threat of a radiological attack by working with sister Institutes within NIH and agencies throughout the Federal Government. Lessons learned through this and subsequent workshops will be used to develop research programs to support methods to protect against and treat a wide range of short- and long-term radiation-induced injuries resulting from a radiological or nuclear attack.

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